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Perspective

Ras Farnesyltransferase: A New Therapeutic Target

Daniele M. Leonard*

Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105

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Introduction

Prenylation of proteins with polyisoprenoids is a functionally important post-translational modification. Prenylation has been well characterized in mammalian cells[1,2] and has been shown to play a major role in cell proliferation of both normal and cancerous cells. Earlier studies had shown that prenylation occurred on certain fungal mating factor peptides.[3,4] It was found that a 15-carbon isoprenoid, farnesyl, was covalently linked to the cysteine sulfhydryl of the protein forming a thioether bond. The functional significance of this post-translational modification was not well understood, but it was found to be stable and an important component of the active mating factor.

The study of the effects of compactin, an inhibitor of the isoprenoid biosynthesis (Scheme 1), on mammalian cell growth and morphology led to the discovery that mammalian proteins could also be prenylated. It was shown that if cells were treated with a high concentration of compactin, cell growth was inhibited;[5,6] however, addition of mevalonate could reverse the effect of compactin, indicating that mevalonate or its metabolite could play an important role in cell growth. Treatment of cells with tritiated mevalonate led to the incorporation of the label into cellular proteins.[7]

The nuclear protein lamin B was the first mammalian protein identified to undergo prenylation.[1,2] Parallel studies on the a-factor mating peptide of *Saccharomyces cerevisiae* showed that this protein became farnesylated.[8] Upon examination of the peptide sequences of lamin B (CAIM) and the a-factor (CVIA), a CAAX motif was observed at their carboxy terminus, where C is a cysteine residue, A is any aliphatic amino acid, and X could be variable. The presence of the CAAX motif was then identified as being the critical moiety involved with the addition of the isoprenoid in the proteins. Such an observation led to the study of the CAAX motif contained in other proteins and investigations of whether they underwent prenylation.

A series of proteins have been identified and shown to undergo prenylation: the nuclear lamins,[9] small guanosine nucleotide-binding proteins of the Ras family,[10] such as Ras, Rho, Rab, the γ-subunit of heterotrimeric G-protein rhodopsin kinase,[11] and γ-transducin.[12,13] Prenylation can occur via a covalent attachment of either a 15-carbon farnesyl moiety or a 20-carbon geranylgeranyl moiety to the cysteine of CAAX motif-containing proteins. The latter, geranylgeranylation, is found to be responsible for the prenylation of 80−90% of the prenylated proteins.[14] Lamin B, the fungal mating factors, γ-transducin, and Ras proteins are farnesylated.

The discovery that Ras proteins are modified by the farnesyl group and that this transformation is required for the oncogenic forms of these proteins to transform cells[10,15,16] has led to intense study of the role of post-translational prenylation in proliferative diseases. This Perspective will discuss the Ras protein family with particular emphasis on Ras farnesyltransferase. Inhibitors of Ras processing have emerged as a novel class of pharmaceutical agents of therapeutic utility in a range of abnormal proliferative diseases.

Ras Proteins

Ras proteins are plasma membrane-bound GTP-binding proteins that play a role in mitotic signal transduction.[17] Mutations that activate Ras result in uncontrolled cell growth and play an important role in malignant transformations.[18]

Three mammalian Ras genes have been identified[19−25] and designated *H-Ras-1*, *K-Ras-2*, and *N-Ras*. Two

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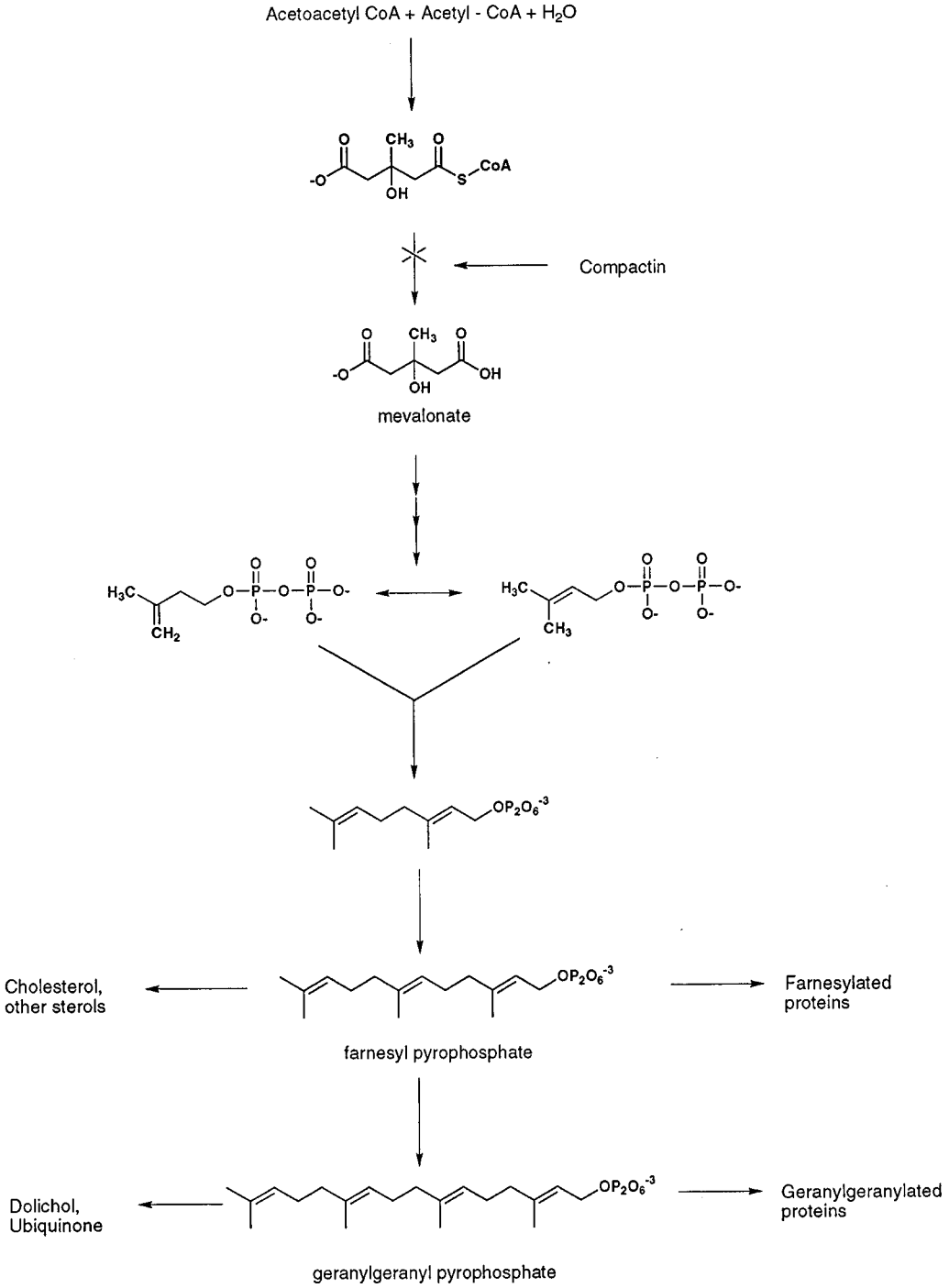
Scheme 1. Isoprenoid Biosynthesis



other genes, *H-Ras-2* and *K-Ras-1*, have been identified and characterized in rats and humans.[19,20,26,27] Each of the three functional Ras genes have been cloned and sequenced in human[24,25,27−36] and encode highly related proteins known as p21.[37]

Ras p21 proteins contain 188 or 189 amino acids (Chart 1). The first 85 amino acid residues of mammalian p21 Ras proteins of known sequence (human H-Ras-1, K-Ras-2, and N-Ras, rat H-Ras-2, and mouse K-Ras-2 and N-Ras) are identical. The next 80 amino acid residues differ slightly (85% homology between any pair). The rest of the protein is a highly variable region except for the last four amino acids where the CAAX motif is present in all members of the Ras family.

The p21 Ras protein is synthesized in the cytosol and is localized to the inner plasma membrane only after it undergoes a series of post-translational modifications including farnesylation. The activity of the Ras p21 is dependent on its membrane localization and is therefore dependent on farnesylation for its activity. Mutants of oncogenic Ras which are not modified at the C-terminus are not capable of transforming cells.[38]

Farnesylation of the Ras p21 protein is followed by the proteolytic cleavage of the three terminal amino acids, AAX[39−41] and methyl esterification at the new C-terminal cysteine residue by a protein methyltransferase, as discussed later (Scheme 2).[42,43] The next modification is the acylation with palmitic acid of cysteine residues located upstream of the farnesylated cysteine[186] (Cys^{181} for N-Ras and Cys^{181} and Cys^{184} for H-Ras) (Scheme 3). Palmitoylation increases the binding affinity of Ras proteins to the cell membrane, and

Chart 1. Sequences of Human Ras Proteins

H-Ras-1:
^{1}MTEYKLVVV^{10}GAGGVGKSAL^{20}TIQLIQNHFV^{30}DEYDPTIEDS^{40}YRKQVVIDGE^{50}TCLLDILDTA^{60}GQEEYSAMRD^{70}QYMRTGEGFL^{80}CVFAINNTKS^{90}F
EDIHQYREQ^{100}IKRVKDSDDV^{110}PMVLVGNKCD^{120}LAARTVESRQ^{130}AQDLARSYGI^{140}PYIETSAKTR^{150}QGVEDAFYTL^{160}VREIRQHKLR^{170}K
LNPPDESGP^{180}GCMSCKCVLS

K-Ras:
^{1}MTEYKLVVV^{10}GAGGVGKSAL^{20}TIQLIQNHFV^{30}DEYDPTIEDS^{40}YRKQVVIDGE^{50}TCLLDILDTA^{60}GQEEYSAMRD^{70}QYMRTGEGFL^{80}CVFAINNTKS^{90}F
EDIHHYREQ^{100}IKRVKDSEDV^{110}PMVLVGNKCD^{120}LPSRTVDTKQ^{130}AQDLARSYGI^{140}PFIETSAKTR^{150}QGVDDAFYTL^{160}VREIRKHKEK^{170}M
SKDGKKKKKK^{180}KSKTKCVIM

N-Ras:
^{1}MTEYKLVVV^{10}GAGGVGKSAL^{20}TIQLIQNHFV^{30}DEYDPTIEDS^{40}YRKQVVIDGE^{50}TCLLDILDTA^{60}GQEEYSAMRD^{70}QYMRTGEGFL^{80}CVFAINNSKS^{90}F
ADINLYREQ^{100}IKRVKDSDDV^{110}PMVLVGNKCD^{120}LPTRTVDTKQ^{130}AHELARSYGI^{140}PFIETSAKTR^{150}QGVEDAFYTL^{160}VREIRQYRMK^{170}K
LNSSDDGTQ^{180}GCMGLPCVVM

although not thought to be essential, the functional consequences are being investigated. In one study,[15] cell lines overexpressing oncogenic H-Ras with mutations at Cys[181,184] caused inhibition of palmitoylation which inhibited membrane binding but did not effect cell transformation. However, in a microinjection experiment in *Xenopus* oocytes, it was found that the palmitoylation of H-Ras increased its membrane anchorage and signaling response.[44] Recently the purification of the protein palmitoyltransferase from rat liver was reported.[45] This finding should allow for further study of the function of the palmitoylation of Ras proteins, particularly the other members of the Ras family. The K-Ras-2 protein is not palmitoylated; however, upstream of the Cys[186] there is a polybasic region characterized by the presence of eight lysine residues (Chart 1), which could be responsible for increased affinity with the cell membrane, by interacting with negatively charged membrane groups.[15,46] Whether palmitoylation inhibitors will find therapeutic utility is really not known.

The Ras p21 proteins, once localized to the cell membrane, bind GTP and GDP and possess intrinsic GTPase activity (Scheme 4). Like other GTP-binding proteins, Ras proteins are active when bound to GTP and inactive when bound to GDP.[47,48] The normal protein exists mostly in the inactive GDP-bound form, and growth signals trigger the formation of the active GTP-bound form. The inactivation phase is regulated by the GTPase-activating protein Ras (GAP) and by the GAP-related domain of type-1 neurofibromatose gene product (NF1) which causes the conversion of Ras protein to the GDP-bound form via hydrolysis of bound GTP.[49]

Oncogenic Ras Proteins

Ras p21 proteins were originally identified as an oncogene product, and examination of genomic DNA from human tumors and cell lines showed that they contained the activated Ras genes. Mutations in naturally occurring Ras oncogenes have been localized at residues 12 (e.g., bladder carcinomas),[50−52] 13 (e.g., myeloid leukemia),[53] 59 (e.g., leukemia),[54,55] and 61 (e.g., lung carcinomas).[36,56,57]

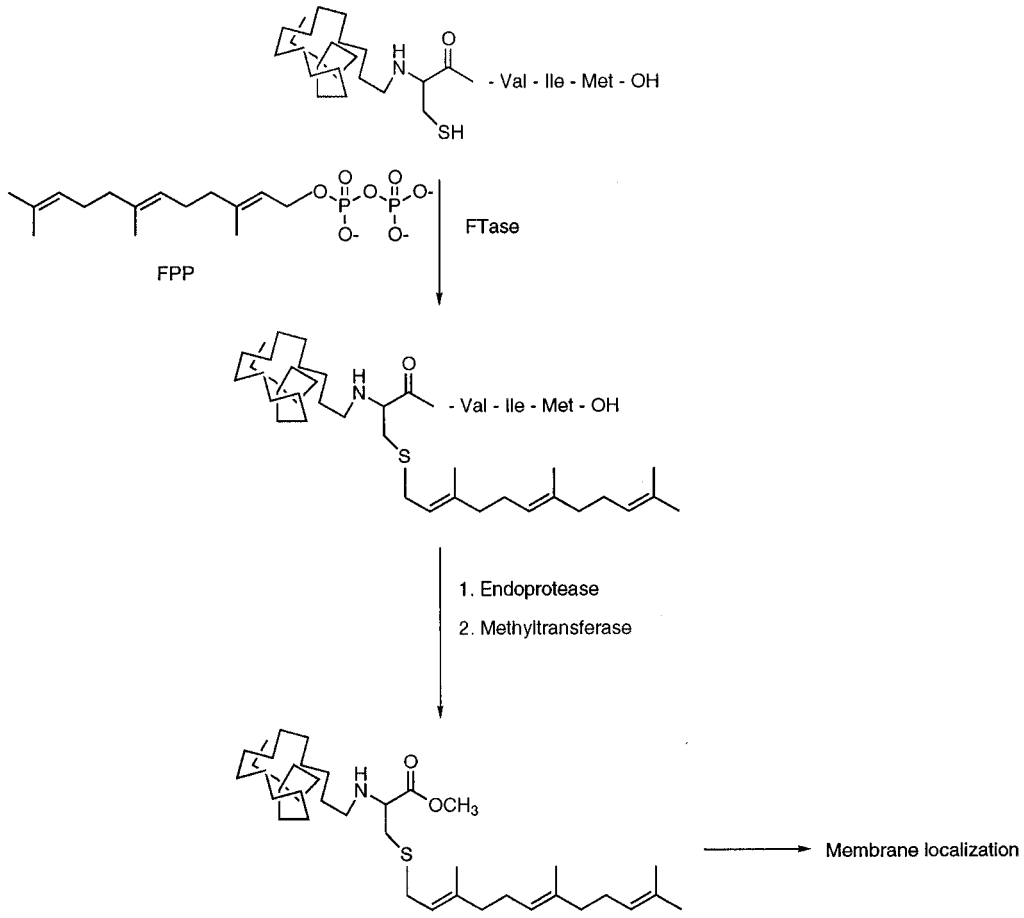
Ras gene mutations have been identified in a variety of human tumors (Table 1) and the point mutations are different for different tumor types.[58,59] In human cancers, the mutation at residue 12 is the most commonly found (Table 2). The glycine residue 12 has been found to be mutated to Ser, Cys, Arg, Asp, Ala, or Val.[58−60]

Human cancer is often characterized by the presence of multiple oncogenic proteins, and indeed Ras mutations have been found in conjunction with other proteins mutations such as p53 and APC.[61] It will be of interest to study further the importance of the Ras mutations and to determine if the Ras farnesylation inhibitors will be efficient at inhibiting cellular proliferation in the presence of these other mutated proteins.

Ras-Related Proteins

The Ras p21 protein is one of the members of the Ras superfamily of GTPases which also includes Rho/Rac, Rab, Ran, and ARF (ADP-ribosyl factor) proteins. These proteins are characterized by common structural fea-

Scheme 2. Post-Translational Modifications of Ras Proteins



tures (at least 30% homology) including the presence of a consensus motif for GTP binding, and they have related functions.[62−64]

The Rho/Rac proteins are involved, in particular, in the morphogenesis and cytoskeletal organization, protein kinase cascades, and transcriptional regulation. Rho/Rac act downstream of Ras in the signaling cascade, and the link between Ras and Rho may be indirect (Scheme 5).[65]

The Rab and ARF proteins are involved in the assembly, loading, and targeting of vesicles to appropriate cellular compartments.[65] ARFs are cytosolic proteins, while Rabs are localized in specific membrane pods. The Ran proteins are associated with localization of proteins into the nucleus.[66]

All the above proteins are required to be in the GTP-bound state in order for them to be active. They are all involved in an intracellular signaling cascade. So far, Ras and Rho/Rac have been shown to be present in the same signaling cascade. All of the Ras GTPase proteins are also capable of interacting with other cytosolic proteins. As more research is carried out, it will be possible to obtain a better understanding of the role of each protein and their importance in cellular signaling and functioning.

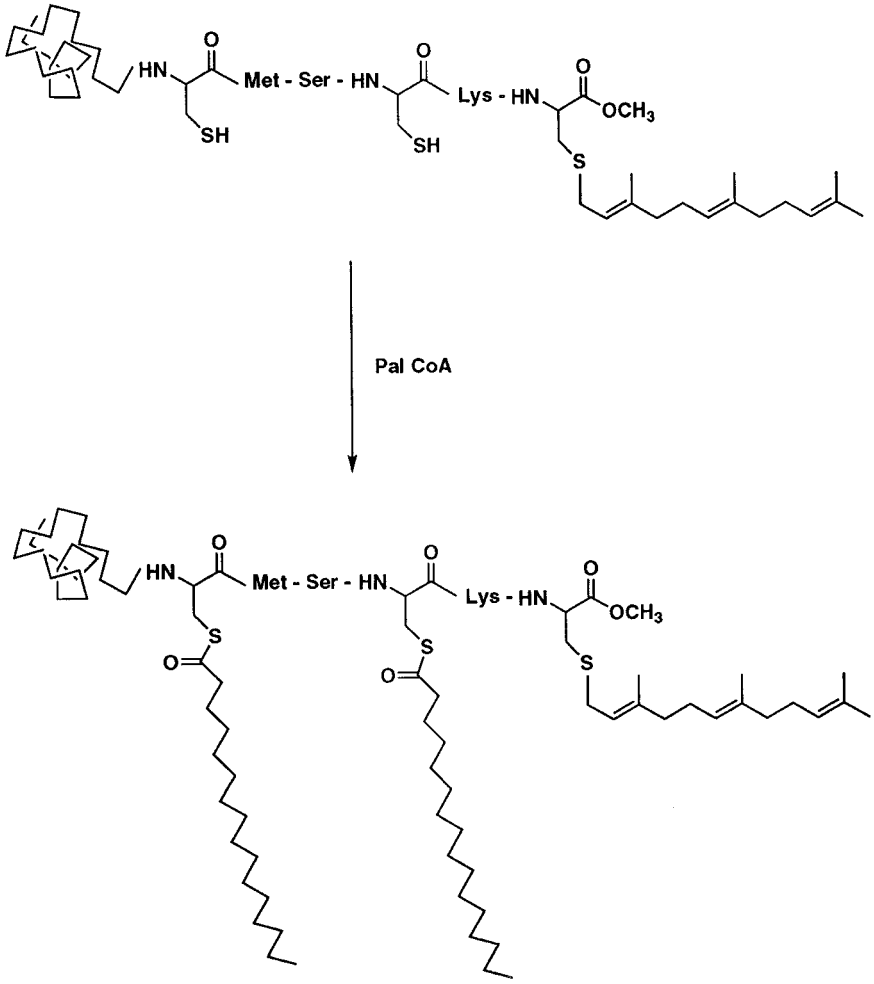
Although inhibition of Ras farnesylation is the target of the FTase inhibitors, there is evidence that other members of the Ras family such as Rho are also affected (see section on FTase inhibitors) and may be important in mediating the antitumor activities of Ras farnesylation inhibitors.

Ras Protein Signaling

The Ras proteins play key roles in cellular processes such as cell proliferation and differentiation.[47,48,67] Ras proteins are involved in intracellular signaling from receptor tyrosine kinases which results in the activation of a phosphorylation cascade (Scheme 4).[68] The Ras protein acts as a "switch" which converts signals from tyrosine kinases to serine/threonine kinases. Upon binding of a growth factor to its receptor (such as FGF, PDGF, NGF, EGF, insulin), dimerization of the receptor occurs, leading to the activation of the receptor tyrosine kinase followed by intermolecular cross-phosphorylation. The phosphorylated tyrosine provides a binding site for the SH_2 domain of a protein, Grb2, which is complexed through its SH_3 domain to a second protein, "Son of Sevenless" or SOS. The SOS protein becomes phosphorylated, activating the Ras protein by becoming GTP bound. The activated Ras associates with serine/threonine kinases, the best characterized being Raf-1, which is recruited to the plasma membrane where Raf-1 is subject to activation by an unknown mechanism.[69,70] Activated Raf-1 will phosphorylate MAP kinase kinase (MEK), which will activate the mitogen-activated protein kinases (MAPKs), $p44^{MAPK}$ and $p42^{MAPK}$, known as extracellular-signal-regulated kinases 1 and 2 (ERK1 and ERK2).[71] The MAP kinases translocate to the nucleus where they can phosphorylate and activate transcription factors, such as c-jun and c-fos, leading to cell proliferation and differentiation.

Further work to elucidate the functional role of Ras has shown that the above linear pathway is just one in which Ras is participating. For activation, it has been

Scheme 3. Palmitoylation of Ras Proteins



Scheme 4. Signal Transduction of Ras Activation

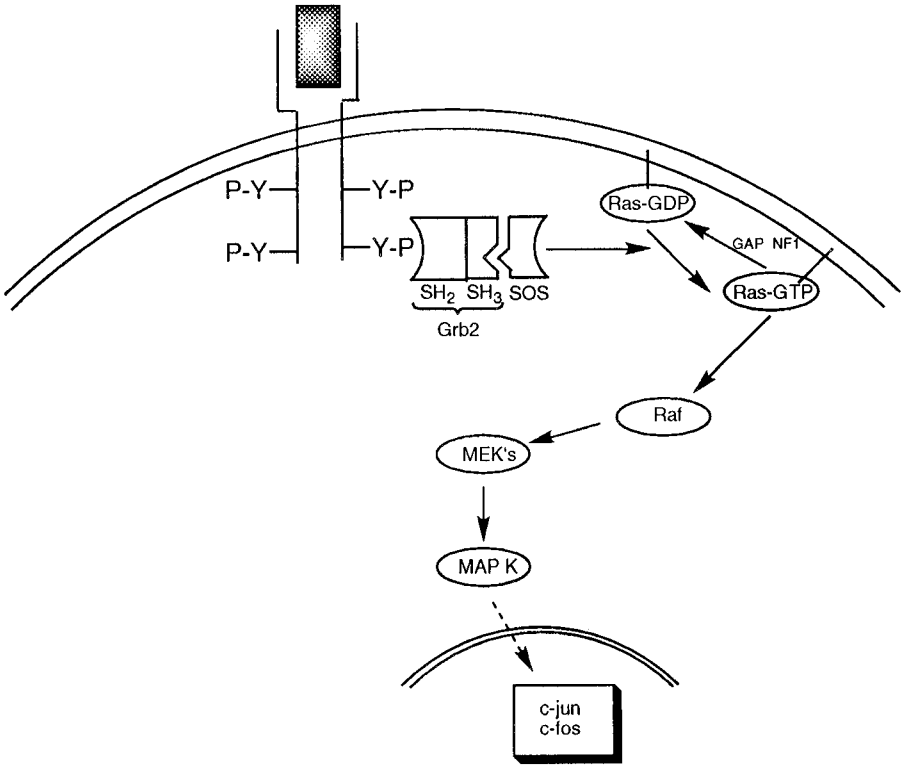


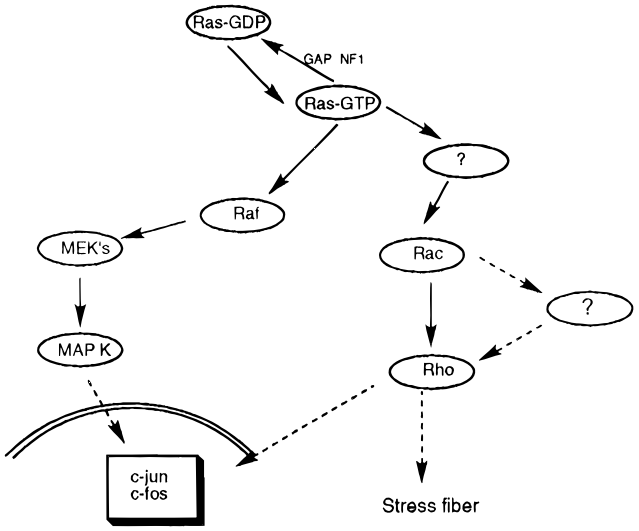
Table 1. Occurrence of Ras Proteins in Human Tumor Cells[58,59]

tumor type	predominant Ras gene	occurrence of Ras gene (%)
pancreas	*K-Ras*	80−90
colon	*K-Ras*	30−60
small intestine	*H-Ras*	31
lung	*K-Ras*	27−60
prostate	*H-* and *K-Ras*	0−25
liver	*K-* and *N-Ras*	12−26
skin	*H-*, *K-*, and *N-Ras*	0−46
thyroid	*H-*, *K-*, and *N-Ras*	0−60
leukemic	*K-* and *N-Ras*	6−40

Table 2. Ras p21 Mutations in Human Cancers

tumor type	p21 position 12 mutation (%)
pancreas	81
colon	38
lung	19
thyroid	22

Scheme 5. Ras Signaling Pathways



found that not only growth factor receptors can activate Ras, but under some circumstances receptors with a serine/threonine kinase domain, such as TGF-β,[72,73] cytokine receptors with no intrinsic kinase such as interleukin-2, -3, and -5,[74] and G-protein-coupled receptors such as the receptors for thrombin[75] and angiotensin-2,[76] can also activate Ras proteins.

Multiple downstream effectors have also been identified which may lead to alternate pathways: they include ral-guanine nucleotide dissociation stimulator,[77−79] phosphatidylinositol 3-OH kinase,[80] the ζ isoform of protein kinase C,[81] and rin-1.[82] The significance of the interaction of these proteins with Ras is still unknown. Two potential models of effector activation have been proposed:[83] a 'recruitment' model and an 'allosteric activation' model. In the 'recruitment' model, the Ras protein, anchored in the plasma membrane, binds to its cytosolic effectors resulting in their activation, as proposed for the Raf protein. In the "allosteric activation" model, the Ras protein interaction with the effector protein will cause in the latter a conformational change which will result in activation. Both mechanistic models probably operate with different effectors within the cell, and more studies are required for a full understanding of the mechanism of the Ras protein binding to its different effectors and their subsequent activation.

A second pathway has been proposed. The above pathway going through Raf is referred to as the MAP kinase pathway, by which gene expression can be regulated. The Ras protein was also found to play a role in the control of the actin cytoskeleton organization, the pathway being referred to as the cell morphology pathway. In order to understand at which point these two pathways are diverging, it was shown that Raf activation was not sufficient to mediate the Ras-induced cell morphology pathway, indicating that the pathways are different directly downstream from the Ras protein.[84] The Rac protein was identified in the second pathway as being activated by the Ras protein.[85] The Rho protein, a member of the Ras family, was shown to act downstream to Ras and was shown to be responsible for stress fiber formation of the cell,[70] suggesting that Rho could act downstream of the Ras protein (Scheme 5).

While the guanine nucleotide interaction clearly regulates the on/off state of Ras as a signal transducer, the post-translational modifications of Ras p21 proteins allowing membrane localization are of utmost importance, in particular, farnesylation which has been shown to be obligatory to Ras p21 activity, but not the following two steps.[86,87] As mentioned previously, the oncogenic forms of the Ras proteins are one of the most common aberrations in human cancers; therefore, inhibitors of Ras p21 farnesylation are of great interest as a potential class of new antitumor agents.

Ras Protein Structure−Activity Relationship

The Ras protein cycles between an inactive GDP-bound state and an active GTP-bound state. The structure/conformation of the Ras protein is different for these two states. The changes in conformation allow for the GTP-bound Ras to interact with its different effector molecules. The changes occur mainly between two regions: switch I (residues 30−38) and switch II (residues 60−76).[88−90] The switch I region contains some of the residues which interact with the various effectors since residues 32−40 are part of what is referred to as the effector region[91] and is believed to be important for the biological activity. The switch II region can interact with the exchange factors, GAP and NF-1, but its role for the activity of the Ras protein is not as well defined.[92] However, as will be shown, key amino acids throughout the protein can modify Ras protein functioning.

The Gly^{60} to Ala mutation was found to decrease GTPase activity of H-Ras, but it did not affect the binding of either GDP or GTP nor the affinity for GAP or NF-1.[92] However, binding of H-Ras to Raf-1 was decreased as well as binding to Ral-GDS.[92] Although all the effectors interact with the Ras protein in the GTP-bound state, the residues on H-Ras for the interaction are different for each effector.[92]

Mutations at residues 17 (Asn for Ser) and 57 (Tyr for Asp) caused inactivity of the Ras protein, in particular in the protein with the position 17 mutation. However the Ras protein could still bind GTP but was unable to activate the signaling pathway.[93] In the wild type Ras protein Ser^{17}-Asp^{57} were found to interact with Mg^{2+}.

Residues 32−42 are highly conserved among the Ras proteins and are important for biological activity. For example, the mutations at Tyr^{32} to Phe and Tyr^{40} to Lys caused a loss in biological activity of oncogenic Ras in

PC12 cells; however, the Trp mutation at both positions did not cause any change in activity.[94] Therefore, these two positions favor the presence of an aromatic group while the 32 residue also requires an hydrogen donor. The latter could play an important role in the change of conformation between the GPD- and GTP-bound forms.

Other regions in the Ras protein are reported to be dispensable: 64−73, 96−105, 123−133, and 165−185.[95] Mutations at positions 12, 13, 61, and 63, commonly found in several tumor types, were found to disrupt GTPase activity, locking the Ras protein in its active form which leads to oncogenicity.

Farnesylation by the Enzyme Farnesyltransferase

As shown in Scheme 2, Ras p21 proteins undergo farnesylation as a first step of the post-translational modifications, and it has been shown to be a required modification for membrane localization.

Prenylation, defined as the attachment of an isoprenoid moiety onto a protein, falls in two types: farnesylation and geranylgeranylation. Both are similar in their behavior although specificity for the one prenylation over the other is determined by the C-terminal sequence of proteins. Proteins with the CAAX motif where X is either methionine, serine, glutamine, or alanine undergo farnesylation while proteins with X as leucine are geranylgeranylated. The processes are catalyzed by the enzymes farnesyltransferase (FTase) and geranylgeranyltransferase (GGTase-I), which transfer a C-15 farnesyl moiety from farnesyl pyrophosphate and a C-20 geranylgeranyl moiety from geranylgeranyl pyrophosphate, respectively. The two enzymes contain two subunits: the α-subunit (48 kDa) shared by both enzymes and distinct β-subunits (46 kDa for FTase and 43 kDa for GGTase-I). Zinc and magnesium are required for activity of both enzymes.[96,97] Substrate recognition has also been shown to be dependent on the upstream region of the C-terminus. In particular, K-Ras-2, which contains a polybasic region, undergoes farnesylation and geranylgeranylation in vitro.[98]

A second distinct geranylgeranyltransferase is known, GGTase-II, and it modifies proteins terminating in C-C, C-C-X-X, or C-X-C. Recently, two distinct farnesyltransferase enzymes were reported in human Burkitt lymphoma Daudi cells.[99] FTase-I corresponds to the previously identified enzyme, while the second isoenzyme, FTase-II, was shown to have a molecular mass of 250 kDa. Both enzymes share a common β-subunit while they have a distinct α-subunit. FTase-II was also shown not to require zinc for peptide binding or inhibition. It will be of interest to further investigate FTase-II with regard to identifying its abundance and location as well as to determine whether it is really important in Ras p21 farnesylation in vivo.

Farnesyltransferase has been identified and characterized from pig[100] and bovine extracts[101] and was purified to homogeneity and cloned from rat brain cytosol.[102,103] More recently, FTase was cloned from human placental cDNA libraries.[104] The amino acid sequence of human FTase was shown to be 95% homologous to the FTase sequenced from rat brain.[104] Geranylgeranyltransferase was characterized[105] and purified from bovine brain cytosol.[106] It has been proposed that the α-subunit common to both enzymes contains the isoprenoid binding site.[107] The distinct β-subunits could contain the binding site for the protein substrate.[107] The identification of the farnesyltransferase enzyme and the importance of farnesylation of oncogenic Ras p21 proteins have led to the search of agents inhibiting this post-translational modification as potential antitumor agents.

During the course of research in this field, important issues have arisen with regards to potency, selectivity for farnesyltransferase versus geranylgeranyltransferase, mechanism of action, cellular penetration, and in vivo efficacy of various inhibitor classes. The ultimate goal will be the proof of concept that indeed a Ras farnesyltransferase inhibitor can reduce tumor growth in vivo, and indeed such evidence is beginning to appear.

Structure of the Enzyme Farnesyltransferase

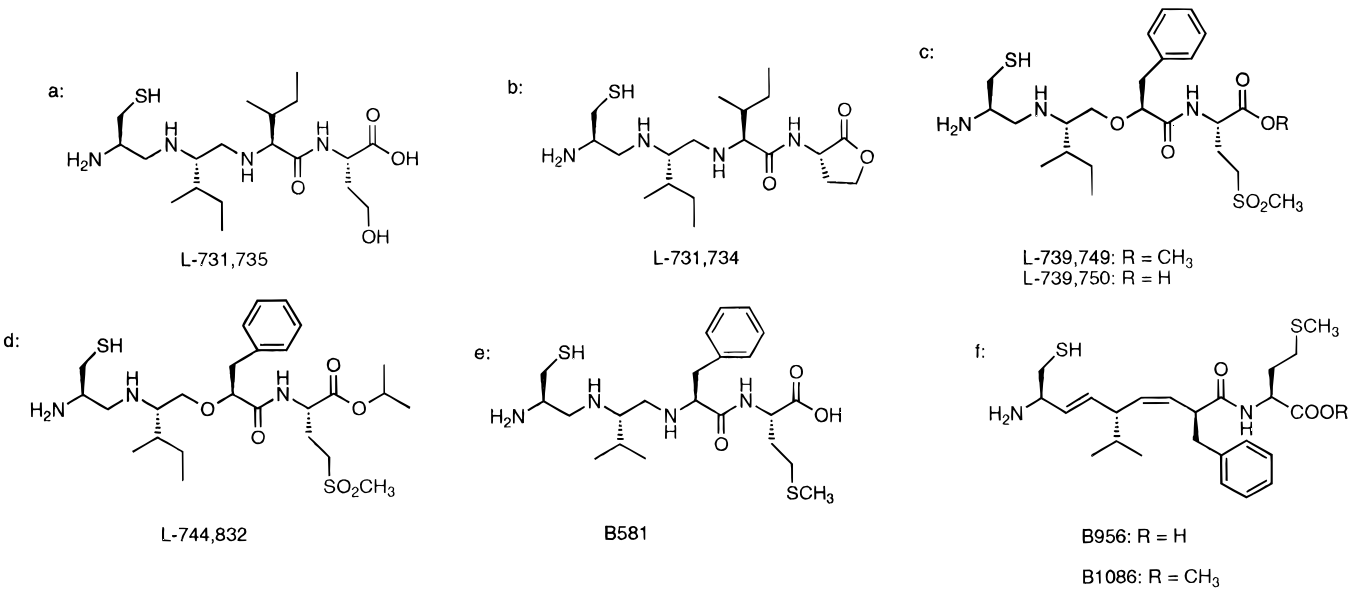
The crystal structure of the enzyme FTase was recently published at 2.25 Å resolution.[108] It was shown that the active site consists of two clefts which are at the junction of a bound zinc ion, which directly coordinates the thiol of cysteine in a ternary complex.[108] One of the clefts was the site for the binding of a 9-mer peptide, which could correspond to the site of binding of the Ras protein, and it was found to be a hydrophilic surface groove near the subunit interface. The second cleft is a region of highly conserved aromatic residues, which could be the binding site for the farnesyl pyrophosphate moiety. It is of interest that this second cleft is of a specific size that would accommodate the farnesyl moiety but could not accommodate the longer prenyl moiety of geranylgeranyl pyrophosphate.[108] With this new information, it will now be possible to carry out studies to elucidate the mode of binding of the various classes of inhibitors, via cocrystallization. Insight into the mechanism of inhibition of Ras farnesyltransferase will likely be forthcoming.

Carboxy-Terminal Protease and Methyltransferase

Farnesylation of the Ras protein is followed by cleavage of the last three amino acids. Recently, two genes in *S. cerevisiae* were identified: *RCE1* and *AFC1*.[109] The Afc1 protein is a zinc protease which cleaves the AAX of yeast a-factor mating pheromone, while Rce1 protein processes both the Ras protein and the a-factor. The Rce1 protein is localized at the membrane which suggests that the prenylated protein is localized to the membrane following farnesylation.[109] The identification of the protein responsible for the proteolytic cleavage offers the possibility of another target for blocking Ras activation, and inhibitors of this enzyme could be an alternative to FTase inhibitors as anticancer agents.

The final post-translational modification of the Ras protein prior to membrane anchoring is the methylation of the carboxyl group of the prenylated cysteine.[25] The methyltransferase responsible for this modification has been identified in several mammalian tissues.[50,110,111] *S*-Adenosyl-L-methionine (AdoMet) is the methyl donor. The same enzyme methylates substrates undergoing either farnesylation or geranylgeranylation.[112,113] Inhibitors against the methyltransferase based on the structure of the prenyl cysteine have been reported. *N*-Acetyl-*S*-*trans*,*trans*-farnesyl-L-cysteine (AFC) inhibited carboxyl methylation of K-Ras protein, at a concentration of 100 μM in rat brain.[114] In H-Ras-transformed rat embryo fibroblasts, 100 μM AFC caused a 60−70% decrease in H-Ras carboxyl methylation. At 10 μM, AFC caused more than 80% inhibition of

Chart 2. Ras FTase Inhibitors Based on the CAAX Motif



chemotactic responses of mouse peritoneal macrophages.[114] Analogues of *S*-farnesylthiopropionic acid have also been reported to be weak inhibitors (K_i > 25 μM) of the methyltransferase.[115] The significance of inhibition of methyltransferase is really not known at present, and study of selective inhibitors in relevant cellular and in vivo models is necessary to advance the field.

Ras Farnesyltransferase Inhibitors

Many classes of farnesyltransferase inhibitors have been discussed in the literature, with most of the work focusing on mimetics of the CAAX box of the Ras protein. Since the designs of these CAAX mimetics were carried out prior to the X-ray structure of the enzyme FTase,[108] the clear mechanism of action and the binding site of these inhibitors are still unknown. The following is an overview of the different types of Ras farnesyltransferase inhibitors and a perspective on the significance of the various classes as therapeutic agents.

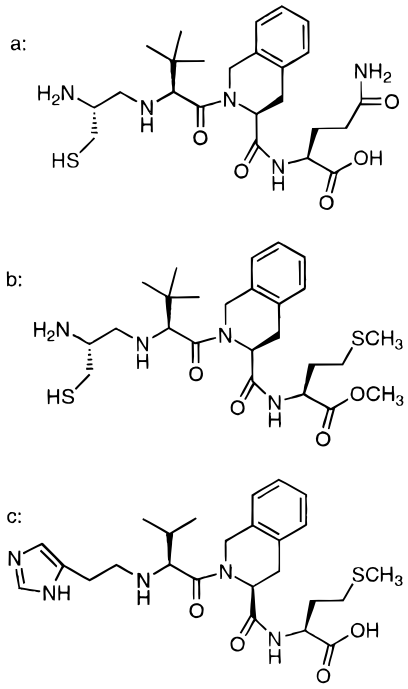
a. Design Based on the CAAX Motif. Initial reports of farnesyltransferase inhibitors were based on the CAAX motif of the natural H-Ras p21 substrate: CVIL. The tetrapeptide itself was found to be a competitive inhibitor with Ras p21.[103] Modifications of the tetrapeptide at the Ile position where the aromatic amino acid phenylalanine was substituted for isoleucine led to a potent Ras FTase inhibitor with an IC_{50} of 25 nM against bovine FTase, and this peptide was not a substrate.[116] Further modifications have also been reported. Introduction of reduced bond isosteres and homoserine for methionine led to L-731,735 (Chart 2a) which has an IC_{50} of 18 nM against bovine FTase. The corresponding homoserine lactone L-731,734 (Chart 2b) had an IC_{50} of 280 nM and was active in cells. L-731,734 inhibited Ras processing in v-Ras-transformed cells and growth in soft agar and reduced Ras farnesylation by 50% in NIH 3T3 fibroblasts in culture at 50 μM.[117,118] Both pseudopeptides were found to be selective for FTase (IC_{50} for GGTase-I > 10 μM).[117] Indeed most groups have made an attempt to increase selectivity for FTase over GGTase-I due to a perceived increased incidence of potential toxicity if both enzymes are inhibited. It is of course possible that greater in vivo efficacy will be demonstrated with 'less selective agents', especially since K-Ras is known to be geranylgeranylated.

L-739,749, the methyl ester analogue of L-739,750 (Chart 2c), is also a pseudopeptide based on the CAAX motif. It is a potent selective inhibitor of bovine FTase, with an IC_{50} of 240 nM.[119] In cells, it was found to inhibit processed Ras by 50% at a concentration between 0.1 and 1 μM; however, it was found to also affect the regulation of actin stress fiber formation.[120] As mentioned earlier, one of the downstream proteins to Ras is Rho. Of particular interest is the Rho-B protein involved in the regulation of various cytoskeletal structures.[121] In general, Rho proteins undergo geranylgeranylation; however, it has been shown that Rho-B can also be farnesylated.[122] Treatment of cells with L-739,749 was shown to inhibit vesicular localization of Rho-B. This FTase inhibitor may then interfere with the farnesylation of Rho-B, leading to the effect seen on the actin stress fiber formation.[123]

Complete inhibition of growth in soft agar was obtained with 10 μM L-739,749 (daily injection, 20 mg/kg, for 5 days) and partial inhibition with concentrations as low as 2.5 μM.[119] However, it had no effect at concentrations up to 10 μM on growth of rat-1 cells transformed by v-Raf and v-mos. In vivo efficacy of L-739,749 has been demonstrated using a nude mouse explant model.[119] There was significant decrease in the average weight of all the Ras-dependent tumors from mice treated with L-739,749, with reductions ranging from 66% for H-Ras-dependent tumors to 51% for N-Ras-dependent tumors.[119] Another CAAX mimetic, L-744,832 (Chart 2d), was found to be active in an in vivo model.[124] In MMTV-v-Ras mice bearing palpable tumors, daily administration of L-744,832 caused complete tumor regression after 2 weeks of treatment (40 mg/kg).[124] These in vivo results, and particularly tumor regression, with various CAAX mimetics are truly significant demonstrating the efficacy of FTase inhibitors as antitumor agents. It will be important to determine the range of tumor types that these compounds are active against for clinical development.

Systematic modifications of the CVFM tetrapeptide

Chart 3. Constrained CAAX Mimetics as FTase Inhibitors



by replacement of the amino-terminal amide bonds led to the inhibitor B581 (Chart 2e),[125] which has an IC_{50} of 0.021 μM against bovine FTase. It was shown to inhibit Ras processing in cells with an IC_{50} of 50 μM. It was reported to be 40-fold selective toward FTase versus GGTase-I in vitro and in NIH 3T3 cells expressing oncogenic (Leu^{61}) H-Ras-CVLS (for FTase) and oncogenic (Leu^{61}) H-ras-CVLL (for GGTase-I).[126] Further modifications led to the inhibitor B956 (Chart 2f) which inhibits H-Ras farnesylation with an IC_{50} of 11 nM.[127] B956 and its methyl ester, B1086 (Chart 2f), inhibit the formation of colonies in soft agar of 14 human tumor cell lines, at concentrations between 0.2 and 60 μM.[127] Tumor cell lines expressing oncogenic H-Ras were the most sensitive to this inhibitor, followed by cell lines expressing mutant N-Ras. The cell lines expressing mutant K-Ras or those without Ras mutations were the more resistant to B956, indicating that this inhibitor is more selective for the inhibition of farnesylation of H-Ras. B956/B1086 at 100 mg/kg inhibits tumor growth of EJ-1 human bladder carcinomas (oncogenic H-Ras-Val^{12}) by about 60%.[127]

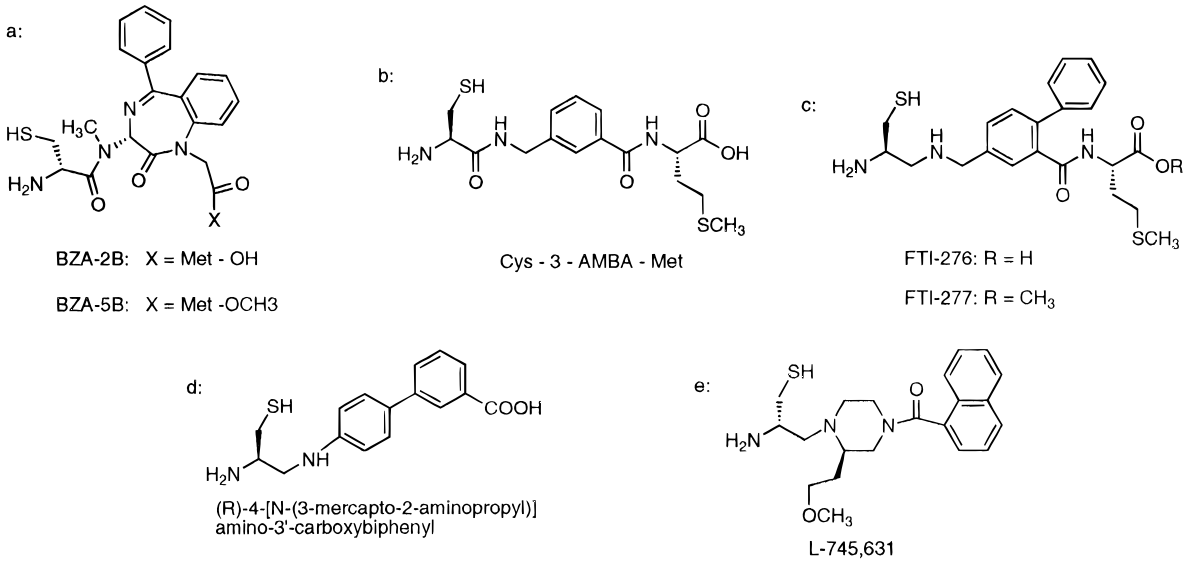
Replacement of the phenylalanine residue in CVFM with 1,2,3,4-tetrahydroisoquinoline-3-carboxylate (Tic) led to increased activity against rat FTase with an IC_{50} of 1 nM for the Tic-containing tetrapeptide compared to 37 nM when phenylalanine was present.[128] This inhibitor is one of the most potent compounds reported to date. Reduction of the amide bond, replacement of the isopropyl group of Val with a *tert*-butyl group, and substitution of Met by Gln led to a compound with an IC_{50} of 2.8 nM against rat FTase and which was also 500-fold selective (IC_{50} against GGTase-I = 1400 nM) (Chart 3a). Retaining the methionine residue as the methyl ester also gave a potent Ras FTase inhibitor (IC_{50} = 85 nM), but it was found to be less selective (IC_{50} against GGTase-I = 200 nM) (Chart 3b). Both compounds were evaluated in vivo in athymic mice implanted ip with H-Ras-transformed rat-1 tumor cells. Both compounds showed activity with a T/C value of 142−154% (ip administration, twice daily for 11 days, 45 mg/kg/injection).[128] Whether these compounds will show activity by using clinically more relevant modes of administration (i.e., subcutaneously (sc), intravenously (iv), or orally (po)) is not known.

Replacement of the cysteine residue by 4-imidazole (Chart 3c) in the above class of inhibitors led to an increase in activity with an IC_{50} of 0.79 nM against rat FTase, and in a clonogenic assay (H-Ras-transformed NIH 3T3 cells) this compound had an IC_{50} of 3.8 μM.[129]

Replacement of the two aliphatic amino acids by a benzodiazepine mimicking a peptide turn, BZA-2B (Chart 4a), gave a potent inhibitor of FTase with an IC_{50} of 0.85 nM against recombinant rat FTase.[130] The corresponding methyl ester, BZA-5B (Chart 4a), was found to be active in cells. At micromolar concentrations, it was able to restore normal growth to Ras-transformed cells.[130] BZA-5B interrupts the MAP kinase activation pathway in H-Ras-transformed cells.[131] A recent report showed that BZA-5B blocks farnesylation of the lamin proteins with an IC_{50} comparable to that obtained for Ras p21.[132] However, it did not interfere with a variety of cellular functions expected to be farnesylation dependent such as cell growth and viability.[132] This report suggests that inhibition of Ras transformation may be specific to malignant cells. Although the above results show no interference with cellular function, the blockade of farnesylation of other proteins should still be of concern. As more potent agents are developed and tested in various in vivo models, it will become of upmost importance to monitor closely if indeed proteins such as lamin-B and transducin are post-translationally modified in the presence of Ras FTase inhibitors as well as and their effects on the viability of the cells.

The two aliphatic residues have also been replaced by a hydrophobic spacer, 3-(aminomethyl)benzoic acid (3-AMBA) (Chart 4b).[133] Cys-3-AMBA-Met inhibits p21 Ras FTase from human colon carcinoma (COLO-205) and Burkett's lymphoma (Daudi) with IC_{50} values of 60 and 120 nM, respectively.[133] Substitution of the two aliphatic residues was also carried out with 4-AMBA and 3- and 4-aminobenzoic acid (3- and 4-ABA).[134] Cys-4-AMBA-Met was 17-fold less potent than the corresponding 3-AMBA analogue, and Cys-3-ABA-Met also showed reduced activity.[134] However, Cys-4-ABA-Met had an IC_{50} of 50 nM. Introduction of functionality such as *N*,*S*-di-Cbz-Cys-3-AMBA-Met-OCH_3 led to compounds which could penetrate NIH 3T3 cells and disrupt p21 Ras plasma membrane association.[135] Substitution at the 2-position of the 4-aminobenzoic acid moiety with a phenyl group led to a potent inhibitor (FTI-276) of Ras FTase with an IC_{50} of 0.5 nM, against human FTase (Chart 4c), and it was 100-fold selective, with an IC_{50} of 50 nM against human GGTase-I.[136] The corresponding methyl ester (FTI-277; Chart 4c) has an IC_{50} of 50 nM against human FTase but inhibits H-Ras processing in whole cells with an IC_{50} of 100 nM[136] and induces accumulation of cytoplasmic nonfarnesylated H-Ras that was able to bind Raf and form a Ras/Raf complex where Raf was not activated.[136] FTI-277 did not inhibit processing of Rap by GGTase-I at concentrations as high as 10 μM.[136] The inhibitor FTI-276 blocked the growth in nude mice of a human lung carcinoma expressing

Chart 4. Ras FTase Inhibitors with Mimetic Replacements of AA in the CAAX Motif



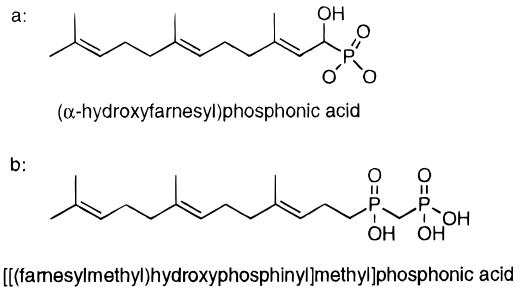
oncogenic K-Ras (50 mg/kg, dosed ip for 36 days).[137] FTI-276 also inhibits oncogenic signaling and tumor growth of NIH 3T3 cells transformed with the Ras oncogene at 20 μM.[136]

Further design led to the replacement of the AAX tripeptide with biphenyl derivatives.[138,139] The analogue (*R*)-4-[*N*-(3-mercapto-2-aminopropyl)amino]-3′-carboxybiphenyl (Chart 4d) was found to inhibit rat brain FTase with an IC_{50} of 50 nM, while it inhibited GGTase-I with an IC_{50} of 100 μM.[138,139] It disrupted H-Ras processing at a concentration of 50 μM (human H-Ras oncogene-transformed Balb/c 3T3 cells).[138,139]

More recently, a piperazine analogue, L-745,631, was reported to suppress tumor growth in nude mice (Chart 4e).[140,141] It was found to be potent against bovine FTase (IC_{50} = 5 nM) and selective (IC_{50} against GGTase-I = 10 μM). It was shown to inhibit Ras processing with an IC_{50} of 0.5 μM and to be noncytotoxic to NIH 3T3 cells at concentrations up to 100 μM. In H-Ras-transfected NIH 3T3 cells, it suppresses tumor growth by 75% at a dose of 40 mg/kg (sc administration).[140,141]

The bioactive conformation of the CAAX-based FTase inhibitors has been studied by NMR spectroscopy. One study suggested that the tetrapeptide of the sequence KTKCVFM adopted a type I β-turn conformation in the bound state.[142] This conclusion was obtained by studying the heptapeptide by NMR spectroscopy in the presence of the FTase enzyme analyzing the transferred nuclear Overhauser effects (NOEs). However activity of the inhibitors where the two aliphatic amino acids have been replaced by 3- or 4-aminobenzoic acid[134] or 3- or 4-(aminomethyl)benzoic acid[133] would argue against a β-turn since, for these tetrapeptide mimetics, the flexibility of the aminobenzoic acid spacer is different than the two amino acids, Val-Phe. In particular, for the 4-aminobenzoic acid spacer, it is not possible for the molecule to adopt a β-turn conformation, and yet it is a potent FTase inhibitor (50 nM).[134] These two different sets of results suggest that the conformation of the terminal CAAX motif needs to be investigated further. The recent disclosure of the structure of the FTase enzyme[108] will hopefully further our understanding of the binding of specific classes of inhibitors and provide insight into the bioactive conformation of the CAAX peptidomimetics.

Chart 5. Ras FTase Inhibitors Based on the Farnesyl Moiety



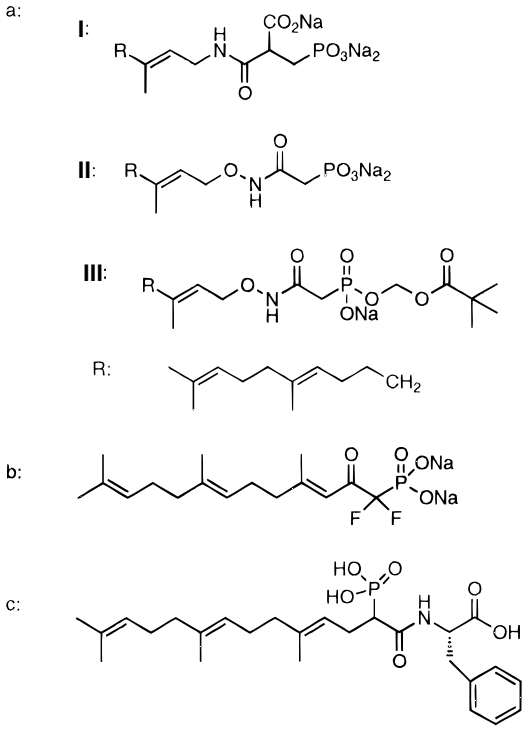
Meanwhile, to investigate the binding sites of the substrates, there have been reports using radiolabeled FPP analogues suggesting that the β-subunit contributes significantly to the recognition and binding of the isoprenoid substrate.[104,143,144] However, there has been no work with elucidating the site for the protein substrate, and at this point interaction with the α-subunit can not be precluded.

b. Design Based on the Farnesyl Moiety. Inhibitors of Ras FTase have also been designed based on the farnesyl moiety of the farnesyl pyrophosphate substrate. This class of inhibitors has attracted less interest due to the possible nonselective effects of competing with farnesyl pyrophosphate which is a substrate for other enzymes (such as squalene synthase).

Two nonhydrolyzable analogues of farnesyl pyrophosphate, (α-hydroxyfarnesyl)phosphonic acid (Chart 5a), and [[(farnesylmethyl)hydroxyphosphinyl]methyl]-phosphonic acid (Chart 5b), were both shown to be competitive inhibitors of farnesyl pyrophosphate with K_i values of 0.0052 and 0.83 μM, respectively.[145] (α-Hydroxyfarnesyl)phosphonic acid inhibits FTase.[146] It was found to inhibit Ras-processing in H-Ras transformed NIH 3T3 fibroblasts at concentrations as low as 1 μM.[146] The selectivity of these analogues for FTase over GGTase-I is not known but would be of interest.

Farnesyl pyrophosphate analogues that are potent and selective inhibitors of FTase were recently described (Chart 6).[147] Farnesyl pyrophosphate analogues **I**−**III** (Chart 6a) are selective inhibitors of FTase in vitro with IC_{50}s of 0.083, 0.075, and 2.61 μM, respectively. The

Chart 6. Farnesyl Pyrophosphate Analogues as Inhibitors of Ras FTase



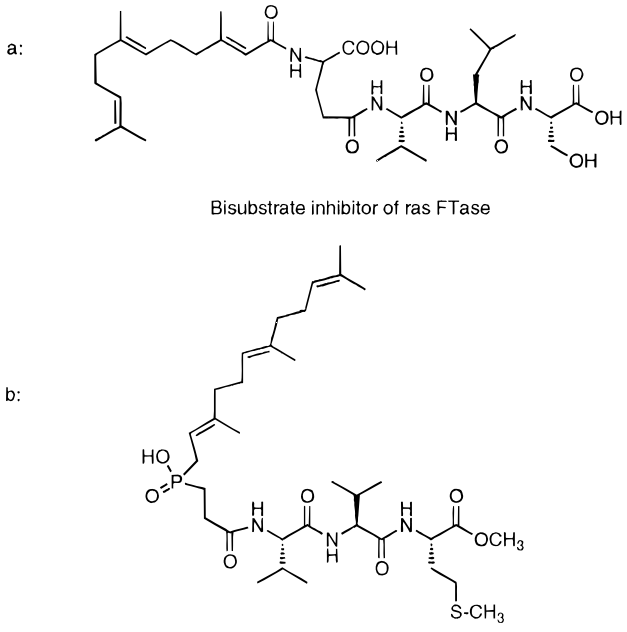
prodrug **III** (Chart 6c) blocked H-Ras-mediated transformation of NIH 3T3 cells at concentrations of 100 μM. None showed toxicity to untransformed cells up to concentrations of 250 μM. This represents the first report of a farnesyl pyrophosphate analogue showing biological activity in inhibiting Ras processing in whole cells.

Mimetics of farnesyl pyrophosphate were also synthesized as possible FTase inhibitors.[148] In particular, incorporation of fluorines at the α-position of the β-ketophosphonic acid (Chart 6b) gave an inhibitor with an IC_{50} of 0.35 μM against pig FTase.[148] Farnesylphosphonate derivatives of phenylalanine have also been reported as potent selective FTase inhibitors (Chart 6c).[149] The compound illustrated in Chart 6c was found to inhibit bovine FTase with an IC_{50} of 0.08 μM.[149] In general, this class of inhibitors has been less potent in cells, and no in vivo activity has been demonstrated for any of these inhibitors.

c. Bisubstrate Inhibitors. Bisubstrate analogue inhibitors of Ras FTase incorporate the structural motifs of both farnesyl pyrophosphate and the CAAX tetrapeptide. The thiol moiety in the CAAX motif was substituted by a carboxylic acid, and the farnesyl chain was covalently attached through a secondary amine on this carboxylic acid to give a Ras FTase inhibitor with an IC_{50} of 0.033 μM (Chart 7a).[150] The selectivity of this inhibitor has not been reported, and it would be of interest to determine whether the replacement of the thiol group by a carboxylic acid group would favor FTase over GGTase-I inhibitory activity and whether these compounds are competitive for FPP or the peptide or both (collected substrate).

Further work led to the phosphinate inhibitor BMS-186511 (Chart 7b).[151] It was shown to inhibit Ras processing in H-Ras-transformed NIH 3T3 cells at concentrations as low as 0.1 μM and showed a higher affinity for FTase versus GGTase-I (>2000-fold).[151]

Chart 7. Bisubstrate Inhibitors of Ras FTase



Similar results were obtained with K-Ras-transformed NIH 3T3 cells. BMS-186511 inhibited [^{3}H]mevalonate incorporation into p21 Ras proteins in a concentration-dependent manner. At 100 μM, the Ras farnesylation was almost completely inhibited in H-Ras- and K-Ras-transformed NIH 3T3 cells. This inhibitor was also shown to be a selective Ras FTase inhibitor (>2000-fold) and had little effect on normal cells.[151]

d. Natural Product Sources of FTase Inhibitors. Several natural product inhibitors of FTase have been reported, but in general these have been less potent than the CAAX-based mimetics. Some are competitive with farnesyl pyrophosphate, including chaetomellic acids,[146,152−154] actinoplanic acid A,[155] and manumycin analogues,[156,157] while other inhibitors such as pepticinnamins[158] are competitive with the Ras peptide (Chart 8). The remaining inhibitors are noncompetitive with neither farnesyl pyrophosphate nor the Ras peptide, and their mechanism of inhibition is not known. Examples of such inhibitors are fusidienol,[159] preussomerins,[160] gliotoxin,[161] 10′-desmethoxystreptonigrin,[162] and cylindrol A (Chart 9).[163]

The potency of this class of Ras FTase inhibitors varies from submicromolar to micromolar (Table 3). One of the manumycin analogues, UFC1-C (Chart 8c), was shown to inhibit the growth of K-Ras-transformed fibrosarcoma at a dose of 6.3 mg/kg, administered intraperitoneally for 5 days from day 0 to day 4.[156]

e. Ras FTase Inhibitors from Compound Library Screening. Another common and successful route to discover novel leads for chemical optimization has been via high-volume screening of compound libraries. Through high-volume screening, PD083176 was identified as a potent selective Ras FTase inhibitor (Chart 10a), with an IC_{50} of 17 nM against Ras FTase and an IC_{50} of 1.25 μM against GGTase-I.[164] PD083176 was shown to inhibit by 30% insulin-induced Ras-dependent maturation of *Xenopus oocytes* at a concentration of 5 pmol/cell. The discovery of this inhibitor was unique in the fact that it was a peptide which

Chart 8. Natural Products as Ras FTase Inhibitors

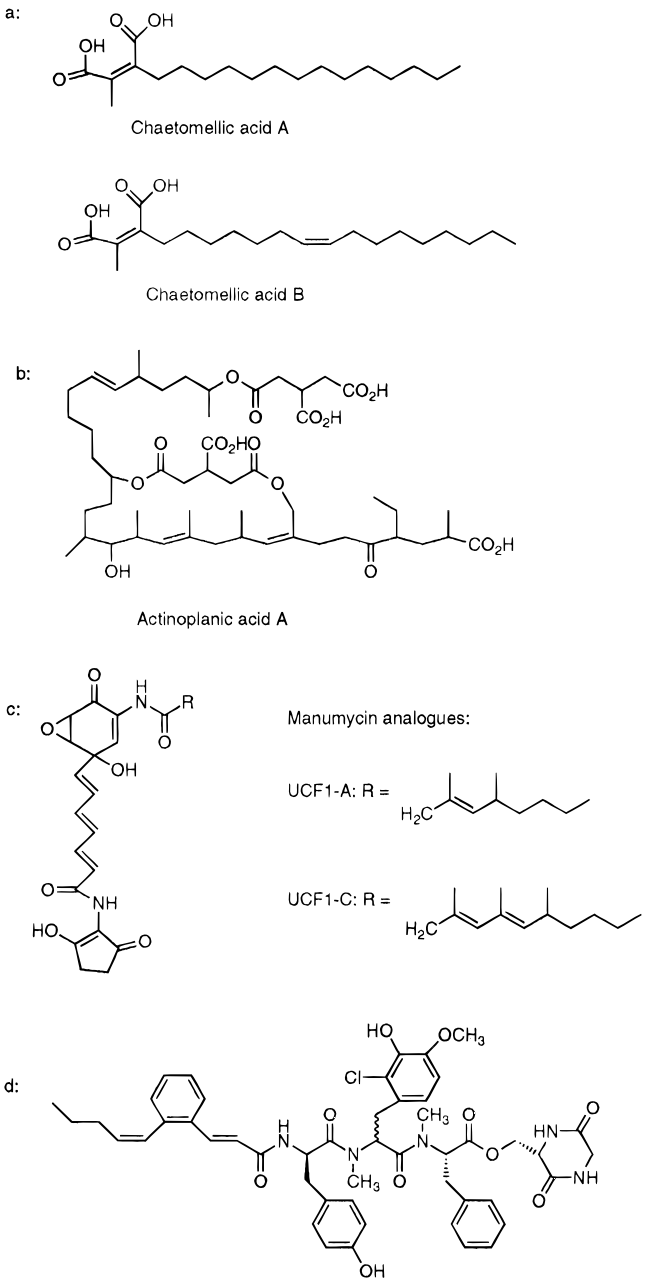


Chart 9. Natural Product Inhibitors of Ras FTase, Noncompetitive for Either Farnesyl Pyrophosphate or the Ras Peptide

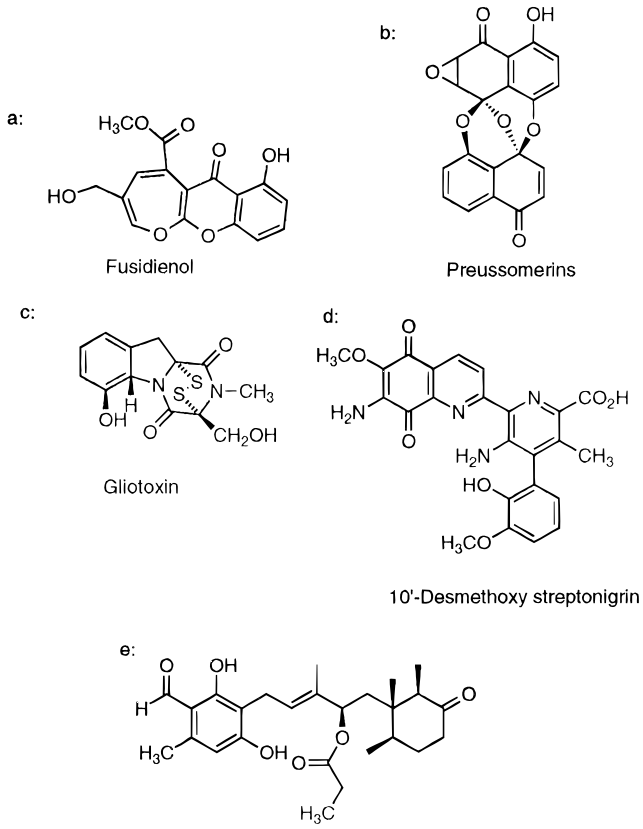


Table 3. Activity of Ras FTase Inhibitors from Natural Product Sources

natural product	IC_{50} (μM)
chaetomellic acid-A	0.06[a]
chaetomellic acid-B	0.19[a]
actinoplanic acid	0.23[a]
manumycin: UCF1-C	5.0[b]
pepticinnamin	0.1[a]
fusidienol 1.0[c]	1.0[c]
preussomerin	1.2[c]
gliotoxin	1.1[d]
10′-desmethoxystreptonigrin	21[d]
cylindrol A	2.2[c]

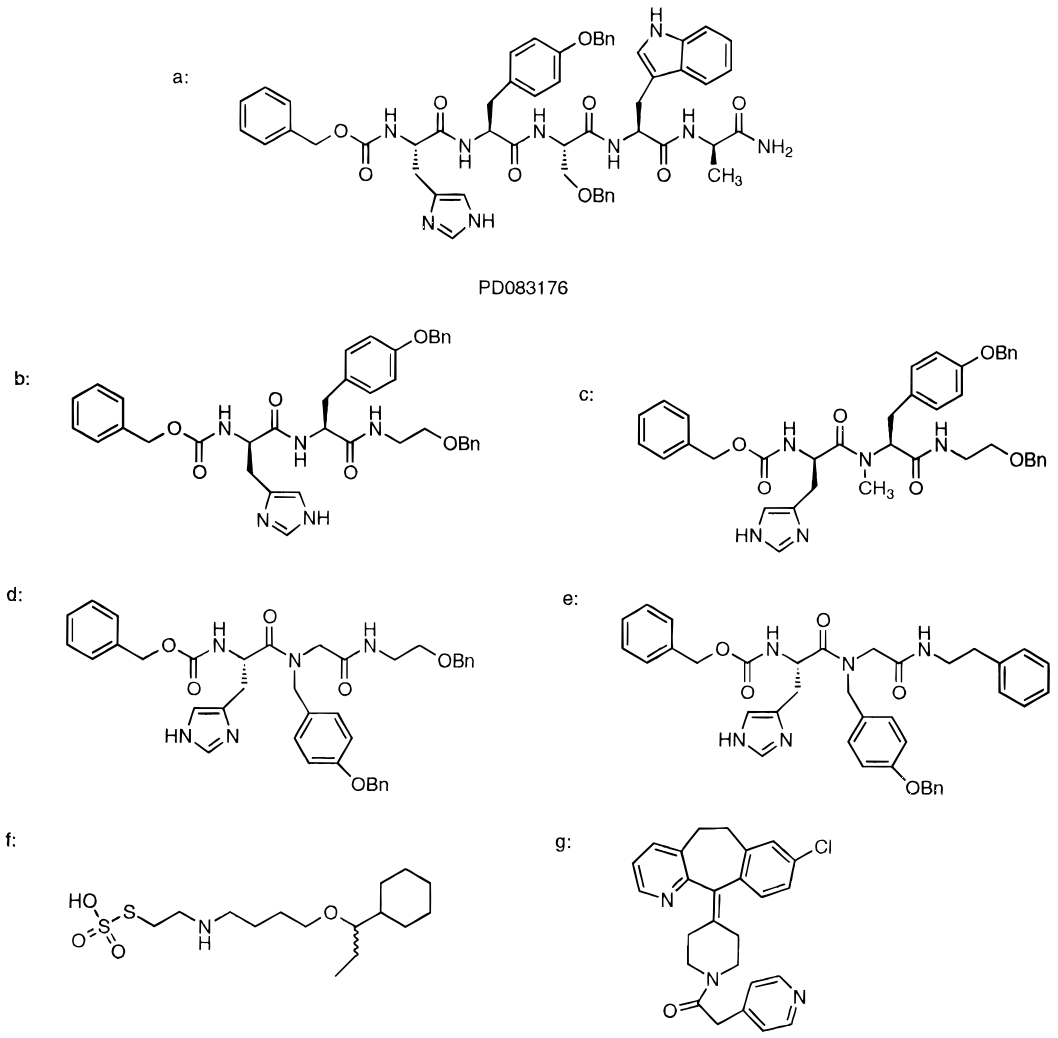
Activity against [a]human FTase, [b]yeast FTase, [c]bovine FTase, or [d]no species of FTase.

lacked the cysteine residue common to most potent FTase inhibitors. Further work has shown that it was competitive against FPP and that the activity against FTase was dependent on the concentration of phosphate ions present in the phosphate buffer.[165]

Since PD083176 lacks any significant cellular activity, probably due to poor cellular permeability, truncation of the pentapeptide was carried out. Deletion of the Trp-DAla moiety from the pentapeptide led to a tripeptide inhibitor with an IC_{50} of 0.42 μM (25-fold loss in activity compared to PD083176).[166,167] Inversion of stereochemistry at the His residue in the tripeptide led to an increase in activity against FTase (IC_{50} = 0.020 μM), and it was found to inhibit Ras processing at a concentration of 25 μM.[166,167] Substitution for the serine residue by *O*-benzylethylamine (Chart 10b) gave a modified dipeptide with an IC_{50} of 0.020 μM, and it was found to inhibit Ras processing in NIH 3T3 cells expressing oncogenic (Leu^{61}) H-Ras-CVLS at a concentration of 5 μM.[166,167]

Further SAR work around the modified dipeptide inhibitor led to an inhibitor, PD154309 (IC_{50} = 0.4 μM) (Chart 10c), with 2-fold improved cellular activity (2.5 μM).[168] Transposition of the Tyr(OBn) side chain to the α-nitrogen was carried out; PD152440 (Chart 10d) was active against farnesyltransferase with an IC_{50} of 0.26 μM and inhibited Ras processing in NIH 3T3 cells expressing oncogenic (Leu^{61}) H-Ras-CVLS at a concentration of 1 μM.[169] Modification of the C-terminal moiety of PD152440 by substituting *O*-benzylethylamine with phenethylamine led to PD161956 (Chart 10e).[170] PD161956 had an IC_{50} of 0.17 μM and inhibited Ras processing in NIH 3T3 cells expressing oncogenic (Leu^{61}) H-Ras-CVLS at a concentration of 0.1 μM; it was found to be 50-fold selective for FTase over GGTase-I. In a clonogenic assay against H-Ras-transformed NIH 3T3 cells, PD161956 had an IC_{50} of 4.4 μM. In vivo evaluation of this modified dipeptide showed that it increased the life span of tumor-bearing nude mice by 35%, at 200 mg/kg (the tumor was implanted ip (NIH

Chart 10. Ras FTase Inhibitors from Compound Library Screening



3T3 cell expressing an oncogenic form of human H-Ras, Gln^{61}-Leu^{61})), and the inhibitor was administered ip, for 2 days on and 2 days off, 5 cycles, 10 treatments).[170]

A series of thiosulfuric acid esters were also identified through a compound library screening as FTase inhibitors (Chart 10f).[171] Their activity against rat brain FTase ranged from 0.2 to 2 μM, and they showed about a 15-fold selectivity for FTase versus GGTase-I. They were shown to inhibit farnesylation of p21 in Ras-transformed cells at concentrations in the 25 μM range.[171]

A series of nonpeptide tricyclic FTase inhibitors were recently published.[172] SCH44342 (Chart 10g) inhibits rat brain FTase with an IC_{50} of 0.25 μM and is reported to be selective (IC_{50} = 114 μM against GGTase-1). In Cos-7 monkey kidney cells transiently expressing H-Ras[Val^{12}], Ras processing was inhibited by SCH44342 with an IC_{50} of 3 μM.[172] It is likely that other classes of inhibitors have been discovered from screening approaches that will be reported in the near future.

Antisense Oligonucleotides

Although most of the research to block the activation of mutant Ras proteins has focused on FTase inhibitors, antisense oligonucleotides have also been designed that block Ras function. The expression of mutant H-Ras can be inhibited by antisense nucleotides which interact with H-Ras mRNA codon 12, where the mutation occurs.[173] These oligonucleotides when adsorbed to polymeric nanoparticles were shown to inhibit the neoplastic growth of HBL100Ras1 in nude mice (sc injection, every 4 days, 52 mg, 4 injections total).[173] Antisense oligonucleotides could also be used against other proteins in the signaling cascade activated by the Ras protein, such as the Raf protein.[174] One advantage for such therapy could be the specificity of the oligonucleotide to the target protein; however, conversely blocking the expression of one of the proteins in the cascade may not be sufficient. In reality the likelihood that Ras antisense will find therapeutic utility is not high in view of the recent discovery of small molecule inhibitors with in vivo activity.

Perspective—Future Directions

Extensive work on the design and synthesis of various classes of Ras FTase inhibitors has been carried out in the last decade. Recently there have been major advances in demonstrating the therapeutic utility of various inhibitors in antitumor models. Compounds from different structural classes have shown high potency against FTase and cellular activity in both mutant Ras- and wild type-dependent tumor cell lines. There are still many issues pertaining to the clinical development of Ras FTase inhibitors as antitumor agents.

Inhibition of the farnesylation of oncogenic Ras processing prevents membrane localization, and therefore biological activity. Is complete inhibition of the Ras activity necessary for inhibition of tumor growth? Can other intracellular proteins restore Ras-like activity? Recent reports indicate that other Ras-related members (R-Ras-2/TC21) exhibit transforming activity comparable to that of the Ras proteins.[175,176] R-Ras-2/TC21 was shown to be a substrate for both FTase and GGTase-I, in vitro.[177] The FTase bisubstrate inhibitor BMS-186511 (Chart 7b) blocked anchorage-dependent and -independent growth of NIH 3T3 cells transformed by H-Ras but not by TC21 or R-Ras-2 and did not affect transformed morphology of normal NIH 3T3 cells by TC21 or R-Ras-2. This study suggests that other proteins are present in the cell which could counteract the effects of Ras FTase inhibitors.

Elucidation of the signaling pathway to the nucleus has helped us understand how the Ras proteins transform cells. However, could an alternate signaling pathway to the nucleus be present and become activated in the presence of a Ras FTase inhibitor? This has been suggested from the work showing that the FTase inhibitor BZA-5B (Chart 4a) can block farnesylation of Ras p21 proteins, but their function and localization were not affected.[132] Further work is needed to confirm the above results and to understand the implications. Already identification of the various Ras protein effectors may elucidate further the signaling pathways where the Ras protein is involved.

Most biological work to study the FTase inhibitors has been carried out with fibroblasts cells. However, epithelial or endothelial cell-based tumors will likely be more relevant to predict clinical efficacy. Some evidence would indicate that Ras transformation may be different in fibroblasts and epithelial cells, where a Raf/MAP kinase pathway may not be sufficient for oncogenic transformation in the latter cell type. Indeed, a Raf-independent pathway involving the Rac and Rho proteins has been proposed.[178] If such alternate pathways can be responsible for cell transformation, it may make the blockade of FTase not sufficient to inhibit tumor growth. Selectivity of inhibition of FTase and GGTase-I could then be nondesirable since most of the proteins such as Rho are geranylgeranylated.

Selectivity and the therapeutic index of Ras FTase inhibitors are two important related issues in this research area. Selectivity is a multifaceted challenge: selectivity of FTase inhibitors for the different Ras proteins, H-, K-, and N-Ras, as well as the other prenylated proteins; selectivity for FTase versus GGTase-I. As various tumors have different distributions of the three Ras oncogenes, should Ras FTase inhibitors block farnesylation of H-, K-, and N-Ras with similar activity? In 80−90% of pancreatic tumors, K-Ras is the prevalent mutant Ras protein;[68] therefore, the FTase inhibitor will need to be potent versus K-Ras if such a cancer is to be targeted. The K-Ras protein is in itself unique. As mentioned previously, a polylysine region present in K-Ras plays a major role in membrane localization and may be sufficient for this anchorage to occur.[179] Also, K-Ras is a substrate for GGTase-I as well as for FTase,[180] suggesting that a nonselective FTase inhibitor may prove useful. As more FTase inhibitors are advanced for studies in various in vivo models, it is hoped that this selectivity issue will be better understood.

Selectivity of the Ras inhibitors for FTase against GGTase-I also needs to be addressed. As mentioned above, it may be desirable to design a selective FTase inhibitor, and indeed most synthetic efforts have focused on this, since most prenylated cytosolic proteins undergo geranylgeranylation (for example, Rho, Rac, Ral, Rap, Rab). However, there has been a report that GGTase-I can farnesylate proteins as well, which could argue that the use of a nonselective Ras FTase inhibitor in vivo may be more efficacious.[181] At the present time there is no clear evidence to suggest how much selectivity is desirable for an FTase inhibitor. Further work is necessary to determine the effects of selective versus nonselective FTase inhibitors, both in vitro and in vivo. Discovery of a GGTase-I selective inhibitor would be useful in this regard.

Selectivity to block farnesylation of Ras p21 over other farnesylated proteins is also an important goal. Inhibition of farnesylation of other proteins is a potential drawback that could lead to undesirable side effects. In particular, the retinal proteins, such as γ-transducin, are farnesylated and are important for retinal signal transduction. One report presents results implicating a defect in protein prenylation in a lovastatin-induced retinal degeneration, suggesting that isoprenylated proteins are required to maintain retinal cytoarchitecture.[182] Such data would indicate the preference for blocking selectively the farnesylation of Ras p21 proteins, although care should be taken in interpreting the lovastatin data due to its mechanism of action.

The selectivity is directly related to toxicity. Indeed, a nonselective agent interacting with various proteins may lead to undesirable side-effects, as discussed above. However, toxicity of Ras FTase inhibitors to normal cells is a key issue. Most reports are indicating selectivity toward mutant cells over normal cells,[124] which is somewhat unexpected. The latter observation does show the need for a better understanding of the mechanism of known Ras FTase inhibitors when acting upon mutant and normal cells. The apparent lack of toxicity of FTase inhibitors against normal cells may indicate that a signaling pathway is activated when wild type Ras is not anchored to the membrane. The different effectors of the Ras protein may not require the latter to be farnesylated for their interaction to occur.

One other important issue is the fact that human tumors have multiple genetic changes. The inhibition of the farnesylation of oncogenic Ras may not be sufficient to stop tumor growth. Alterations in proteins such as p53 may allow for cell growth even in the presence of an FTase inhibitor. A CAAX peptidomimetic closely related to L-739,749 (Chart 2c)[183] was found to inhibit anchorage-independent growth in the human pancreatic cell line PSN-1 and in human breast cell lines MCF-7, MDAMB468, and MDAMB231; however, it did not inhibit growth in the human breast cell lines T47D, BT474, and SkBr3.[183]

Investigation of whether human tumor cell lines would be sensitive to the presence of FTase inhibitors was carried out with L-744,832 (Chart 2d).[61] The panel of tumor cell lines contained some cell lines where Ras mutations were present, while others did not contain

the Ras mutation but only wild type Ras protein. L-744,832 inhibited anchorage-dependent and -independent growth of more than 70% of the tumor cell lines tested. Lack of toxicity to normal tissues was also observed.[61] This study could indicate that in tumor cell lines with wild type Ras protein binding of growth factors could be responsible for cell proliferation. As was shown previously, the Ras protein plays a central role in the signaling cascade, and the inhibitor could be blocking wild type Ras membrane anchorage. These results actually impact the development of FTase inhibitors in a very important way. Concentrating on tumors with only Ras mutations may lead us to miss potential therapeutic efficacy in other types of cancers driven by different oncogenes.

Ras FTase inhibitors have been shown to cause cytostasis of tumor cells. One of the advantages of a cytostatic agent is that it will not be as toxic as conventional anticancer agents which are cytotoxic and cause cell death to both normal and tumor cells. The therapeutic index of a FTase inhibitor may be improved over cytotoxic agents, and in addition combination therapy is worthy of study.

Ras FTase inhibitors could be used in combination with radiosensitization of tumor cells. In one study,[184] treatment of H-Ras-transformed EJ bladder carcinoma cells with the FTase inhibitor FTI-277 (Chart 4c) led to an increase in cell deaths following irradiation. However, radiosensitivity of normal cells was not affected.[184] These results further show that the Ras oncogene can increase the intrinsic resistance of cell to ionizing radiation, as was proposed previously, and this could be an important clinical opportunity.[185]

As mentioned above, most FTase inhibitors have been shown to be cytostatic. However, in one study, L-744,-832 (Chart 2d) was shown to cause tumor regression in MMTV-v-Ras mice, at a concentration of 40 mg/kg, and upon discontinuation of treatment, the tumor reappeared.[124] This result is somewhat unexpected but could suggest that inhibition of cellular FTase activity slows down the Ras-dependent cell transformation, causing some cells to regress. However, in this study it is pointed out that although the Ras oncogene is critical for the formation of the tumor in MMTV-v-H-Ras mice, at least one other genetic change may be necessary for tumor formation since the tumor grows in a random fashion.[124] Since we do not know if this second factor is affected by L-744,832, it is unclear if the tumor regression may not be due to this second factor. The testing of this FTase inhibitor in a tumor model which is solely dependent on Ras for its growth should be useful to elucidate the above results.

Another important consideration in the research on FTase inhibitors is the possibility that they may be substrates for the P-glycoprotein causing multidrug resistance (MDR). The P-glycoprotein allows the efflux of unnatural drugs from the cell. Although not yet known, it will be important to study if indeed MDR can occur with chronic administration of FTase inhibitors. The various classes of inhibitors may have different effects on MDR as well. This is a key area to investigate.

The Ras protein is also being studied for its role in cell death of tumor cells. The MAP kinase pathway and activation of Ras may be involved in the nuclear response which causes apoptosis. For example, tumor necrosis factor (TNF) is cytotoxic to many types of tumor cells but not to normal cells, and Ras activation may play a role in TNF-induced apoptosis.[186] Introduction of molecular antagonists of Ras, the *Rap1* tumor suppressor gene or the dominant-negative *H-Ras* (Asn-17) gene, into H-Ras-transformed 10TEJ cells inhibited TNF-induced apoptosis, suggesting that Ras-activation is required.[186] More research on apoptosis and its mechanism will provide further understanding on the role of the Ras protein.

Some questions about the nature of FTase itself have also been investigated. A recent paper by Vogt et al.[99] reports the identification of a second FTase in human Burkett lymphoma Daudi cells, indicating that there could be two distinct farnesyltransferases. Further investigation is needed on the nature of this putative FTase-II, and it will be necessary to study its selectivity, location, and function.

The potential of Ras farnesyltransferase inhibitors as cancer therapeutics is exciting and challenging. The next few years will see the clinical development of some of these compounds where our understanding of the safety and efficacy in cancer will be elucidated. Farnesyltransferase inhibitors may also have therapeutic utility in other proliferative diseases such as restenosis,[187] psoriasis, endometriosis, and atherosclerosis, and investigation in relevant animal models is an area of considerate importance. The diversity and different selectivities of potent Ras FTase inhibitors reported to date will most certainly advance our understanding of the importance of prenylation in human diseases.

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Biography

Daniele M. Leonard received her B.S. in chemistry from McGill University in 1983, her M.S. in chemistry with Professor H. Alper at the University of Ottawa in 1986, and her Ph.D. in chemistry with Professor G. R. Brown at McGill University in 1990. After postdoctoral work in chemistry with Dr. A. M. Doherty at Parke-Davis, she joined the Parke-Davis Chemistry Department in 1992. Her current position is Research Associate in Chemistry, with research focusing on Ras farnesyltransferase inhibitors.

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